IIAa. CONDITIONALLY ESSENTIAL "VITAMINS"

2. Choline

General - water-soluble;

- choline can be produced in the body if diet contains sufficient protein;
- choline precursor is the essential amino acid methionine;
- a key component of lecithin;
- history: synthesized in 1866; identified as factor preventing fat accumulation in liver of dogs in 1937; biosynthesis pathway identified in 1941; route for incorporation into lecithin identified in 1956;

Nutrition

- sources : lecithin, egg yolks, soy beans, liver, fish, whole grains, legumes, fatty natural foods, cauliflower, cabbage; supplements: lipotropics, B-complex, multi-vitamin, multimineral-vitamin formulations;
- absorption: from duodenum & along entire small intestine;
- storage: higher quantities found in liver; distributed throughout body in cell membranes;
- metabolism: choline forms 10% of lecithin; synthesized from methionine, with help of B-12 & folic acid ; carbohydrate loading increases liver triglyceride synthesis, & increases need for choline-containing lipoprotein envelopes; increased choline necessary during periods of rapid growth (infancy);
- interactions : tricyclic anti-depressants, anti-histamines, & anti-spasmodics interfere wit acetylcholine function & short-term memory;

Functions of Choline

- main function is probably to make methyl groups available for biological reactions;
- part of lecithin (phosphatidylcholine) molecule, important component of all membranes, & main emulsifier (mixing oil & water) in body;
- part of the acetylcholine molecule, an important neurotransmitter;
- participates primarily in the metabolism of fats & nerve tissue;
- prevents deposition of fats in liver; essential for liver & kidney function;
- involved in: digestion, synthesis, & transport of fats to cell membranes in all tissues; metabolism of fats in bloodstream & kidneys;
- probably releases carnitine (required for fat metabolism) from tissue storage; other methyl donors

(betaine, methionine, sarcosine) cannot do this;
- provides methyl groups for carnitine synthesis (made from trimethyl-lysine);
- keeps gall bladder cholesterol in solution, preventing formation of gall stones;
- vital for synthesis of neurotransmitter acetylcholine; maintains integrity of myelin sheath surrounding nerves;
- donates methyl groups (CH3) to make methionine from (toxic) homocysteine, betaine (which stores methyl groups), dimethylglycine (B-15) a metabolic intermediate; & other biological reactions;
- may aid in hormone production;

Quantities

- measurement: in milligrams;
- optimum (SONA) average not yet established
- individual optimum needs to be individually determined;
- minimum (EC RDA) not yet established; choline is beneficial, but not an essential nutrient; can be made from amino acid serine (B-6 required);
- deficiency from lack of dietary lecithin, choline, or precursor amino acid methionine;
- symptoms include: fatty infiltration of liver (steatosis) & damage to liver cells (cirrhosis), nephritis, kidney damage, atherosclerosis, arteriosclerosis, & stomach ulcerations; loss of short-term memory;
- toxicity: "fishy" smell from choline ingestion results from bacteria in gut; choline may cause depression in a few people;

Therapy with Choline

- 500 to 2,000 mg/day may be used
- fat solubilizing;
- patients on intravenous (i.v.) may require choline as part of i.v. nutrient formulation;
- oral administration of choline reduces high blood pressure slightly (may increase vagal tone, dilating arterioles); intravenous choline lowers blood pressure slightly;
- may help improve kidney function
- may help prevent (but not reverse) Alzheimer's disease;
- improves short-term memory in some people;
- helpful in treating tardive dyskinesia, a side effect of anti-psychotic medications;
- may help in Parkinson's disease, Huntington's disease, Tourette's syndrome; Friedreich's ataxia;
- 1,000 - 1,500 mg/day controls manic symptoms in lithium-resistant manic-depressive disorder;
- reduces heart palpitations, dizziness, headaches, ear noises, constipation within 10 day (anecdotal); improves insomnia, visual disturbances, blood flow to eyes (anecdotal);
- more than doubled 3-year survival rate of patients hospitalized for atherosclerosis;

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Recent Findings on N,N-Dimethylglycine (DMG): A Nutrient for the New Millennium

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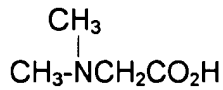
N,N-Dimethylglycine (DMG) has been marketed as a nutritional supplement since 1974 and has seen wide use in both human and animal health fields. Research beginning in late 1978 found that DMG could positively influence the immune response in laboratory animals and humans,[1] boost physical and mental performance in athletes[2] and older people, and enhance cardiovascular function in clinical patients.[3] Other work seems to indicate that DMG can protect the liver, aid in detoxification, reduce seizure activity in some individuals[4] as well as promote improvement in children and adults with autism.[5]

More recently, animal studies done at Clemson University indicate that DMG may have a role in the prevention and treatment of systemic lupus erythematosus (SLE) and melanoma.[6-9] Positive results using DMG have also been seen in a recently completed double blind autism study in children coordinated by the Autism Research Institute.[9] These and other reports are increasing our understanding of the prophylactic and therapeutic use of DMG as a metabolic enhancer and immunomodulator. This article will briefly review previous DMG research, but will focus primarily on the more current research findings.

What is N,N-Dimethylglycine (DMG)?

N,N-Dimethylglycine is a tertiary amino acid, the dimethylated derivative of glycine, and is a natural component of animal and plant metabolism. In the body, DMG is produced in the one-carbon transfer cycle from choline via betaine in an enzyme controlled transmethylation reaction. DMG is a normal, physiologically active nutrient found in low levels in such foods as cereal grains, seeds, beans and liver. In the body, the liver converts DMG into other useful metabolites by a process known as oxidative demethylation (See Figure 1). In this process, the two methyl groups of DMG can be made available for transmethylation reactions via Sulfur-Adenosylmethionine (SAMe), a metabolite that is essential to the production of many vital products of the cell. Mackenzie and Frisell published a paper in the *Journal of Biological Chem* in 1958 where they discussed the metabolism and pathways of DMG.[10] J.W. Meduski, Ph.D., of the University of Southern California has called DMG a "metabolic enhancer" because of the many ways DMG can improve cellular metabolism, enhance oxygen utilization and promote a stronger immune response.[11]



N,N-Dimethylglycine

DMG and the Transmethylation Process

DMG supports transmethylation processes through its ability to give up its methyl groups to help produce Sulfur-adenosylmethionine (SAMe). SAMe is the principle methyl donor in the body and transmethylation involves the reaction whereby a methyl group (CH_3) is transferred from SAMe to another molecule. It is a biochemical process, which is essential to life, health and regeneration of body cells. Many hormones, neurotransmitters, enzymes, nucleic acids (DNA, RNA) and antibodies depend upon the transfer of methyl groups to complete their synthesis. The body detoxifies potentially damaging chemicals and regulates a number of cellular processes through SAMe.[12]

DMG is a key player in the intricate network of methylation and transmethylation in the human body. The major dietary sources of active methyl groups in the human diet are in the form of choline, creatine or methionine. When these methyl donors fail to supply adequate methyl groups, the metabolic role of supplemental DMG takes on profound importance as a provider for methylation for biological methylations. These reactions include methylations of proteins, of nucleic acids and biogenic amines, as well as the detoxification of foreign substances. For example, the generation of the synaptic mediators in the central nervous system (CNS) such as norepinephrine and dopamine requires a methyl group donated by SAMe.

As shown in Figure 1, Dimethylglycine provides methyl groups that can be used to produce the amino acid methionine from homocysteine. This transformation requires folic acid, NAD+, FAD and vitamin B_{12} for its completion. Methionine in turn, is used to produce SAMe.[12]

Therefore, DMG acts as an indirect methyl donor and functions as an efficient "methionine pump" by converting excess homocysteine molecules to methionine. This process keeps the cells of the body in a high state of transmethylation potential, and reduces the concentration of homocysteine in the blood, which can build up when there is a low availability of methyl groups. Kang and coworkers have reported that homocysteine is a potential contributor of vascular disease in humans.[13] Supplementing the diet with DMG may play an important role in keeping homocysteine at acceptable levels while making more SAMe available for transmethylation reactions. In the same way, DMG may play an important role in cardiovascular health.

In addition to generating methyl groups, Dimethylglycine generates two carbon molecules such as sarcosine glycine, serine and the ethanolamines, all of which are beneficial to the cell. For example, glycine functions as an important inhibitory neurotransmitter of the central nervous system.[14] It is used to produce phosphocreatine, a high energy phosphate molecule, used in muscle tissue and in the tissue of the central nervous system.[12]

The metabolic role of DMG as a supplier of both one and two carbon molecules to the cell and its contribution to the formation of SAMe, may explain many of the broad metabolic and therapeutic effects of DMG.

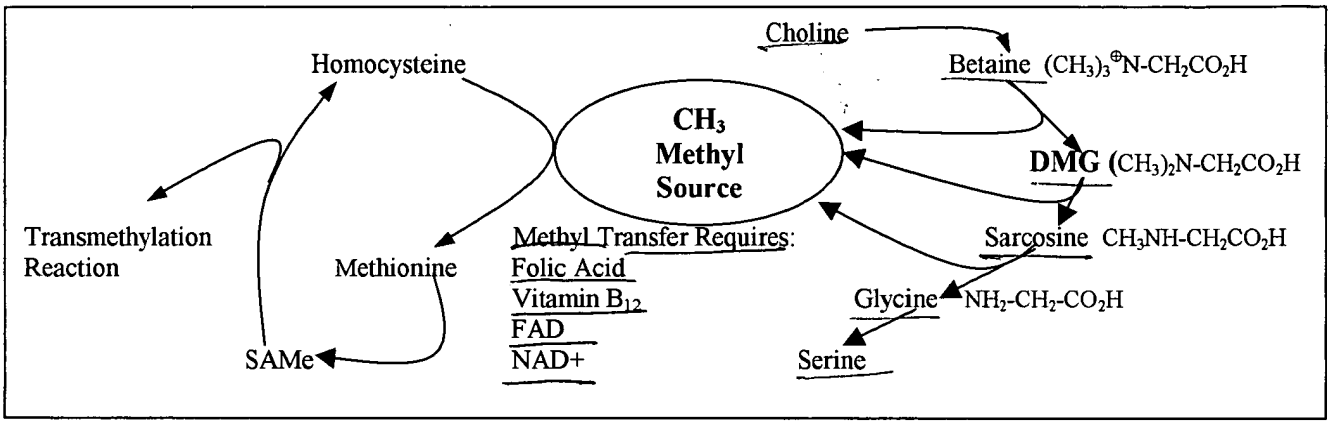


Figure 1: Metabolic Pathway for DMG Showing Methyl Transfer Steps

Possible Mechanisms of Action

DMG may promote health and improve cellular processes by several possible mechanisms.

Table 1: Potential Mechanisms and Resulting Functions of DMG

MECHANISM	RESULTING FUNCTION
Transmethylation	Source of two methyl groups for SAMe
Metabolic Intermediates	Source of sarcosine, glycine, serine, etc.
Immune Response	Modulates T and B cells and their products
Chemical Messenger	Impacts immune modulation and oxidative processes

Wide Ranging Nutritional Applications of DMG

DMG is a versatile nutrient and an intermediary metabolite that can enable a person or animal to function at more optimum mental and physical levels. It aids the body in overcoming a number of adverse health conditions, and is an intricate part of human metabolism. DMG has been used as a nutritional supplement for over 25 years. In a broad sense, DMG protects the body from many forms of physical, metabolic and environmental stress. Table 2 lists the potential beneficial uses for DMG.

Table 2: Potential Areas of Use for DMG

Application	Benefits	References
1. Immune Modulation	Improves Antibody Response Enhance B and T cell function Cytokine Regulation	1, 6-9, 18-20
2. Viral/Bacterial Infections	Enhances Immune Response	1, 20-21
3. Chronic Fatigue Syndrome	Greater Mental Alertness and Energy Improves Immune Dysfunction	3
4. Melanoma	Anti-tumor Activity Prevents Metastasis	6-9, 22-24
5. Lupus (SLE)	Reduces Antinuclear Antibodies Modulates Cytokine Production	6-7, 9, 25
6. Cardiovascular	Reduces Cholesterol/Triglyceride Levels Helps Eliminate Hypoxia Improves Coronary Circulation Decreases Angina Pain	3, 15, 27
7. Athletic Performance	Enhances Endurance Improves Oxygen Utilization Reduces Lactic Acid Buildup Improves Muscle Recovery	28-34
8. Autism/Epilepsy	Improves Verbal Communication/Social Interaction/Lethargy Reduces Seizures	4, 5, 35-37

Earlier Findings

Dimethylglycine was extensively researched in the Soviet Union in the 1960s as part of a formula known as calcium pangamate. Calcium pangamate, incorrectly referred to as "vitamin B_{15}", was reported as being beneficial for athletic performance, cardiovascular function, detoxification, skin problems and liver function, among others. This DMG based-formula was found to aid cardiovascular function by reducing angina, high triglyceride and cholesterol levels, and hypoxia as well as improving circulation to the extremities. Soviet research on this DMG-based formula demonstrated three basic properties.[15]

1. Lipotropic effect (improve liver function)
2. Activation of oxygen metabolism (improve hypoxia and cardiovascular function)
3. Detoxifying action

As reported in US patent 3,907,869, calcium pangamate is defined as the ester formed between Dimethlyglycine and calcium gluconate.[15] The patent clearly reveals that the preparation contained significant amounts of DMG in its free state. Calcium pangamate is not stable to normal digestive processes and would rapidly hydrolyze to DMG when given orally. For all practical purposes, the active moiety in calcium pangamate is in fact DMG. Using this DMG-based calcium pangamate formula, researchers at the Kazan Veterinary Institute in the then Soviet Union reported that it was effective in restoring immune competence in guinea pigs and rabbits exposed to intense X-irradiation.[16] Based on this research, Charles Graber, Ph.D., at the Medical University of South Carolina in Charleston, began to investigate the potential immune protecting and modulating effects of DMG in 1978.[1]

Beginning in 1975, work done on DMG in the United States began to confirm many of the health benefits found in the Russian studies, particularly in the areas of cardiovascular function and sports practice. Initially, DMG was found to significantly improve animal performance. Veterinarians and trainers found that DMG could reduce lactic acid buildup and improve racing performance in both equine and canine events. Several published studies confirmed this. Better endurance and improved recovery time from intensive training and racing were also noted in human athletes.

The clinical and nutritional benefits of DMG were quickly recognized, especially in areas of cardiovascular disease and weakened immunity associated with degenerative conditions, aging and increased stress. DMG was also found to have anti-seizure activity (epilepsy), to provide major improvement in autism, and to reduce toxicity to several potent pathogens.

Immune Response Enhancement

DMG can strengthen the immune system and assist the body in fighting against foreign antigens such as bacteria, viruses and other pathogens. DMG has been shown to significantly influence immune function in a number of *in-vitro* and *in-vivo* models. Charles Graber, Ph.D. and his research team at the Medical University of South Carolina first discovered DMG's role in immune enhancement in 1978.[1,17]

The results of this research, published in 1981 in the *Journal of Infectious Diseases*, centered on three principle findings:[1]

1. DMG invoked a humoral (increased antibody response) in rabbits given a typhoid vaccine, thus demonstrating enhanced B-cell activity.

2. DMG increased T-lymphocyte population in an *in-vitro* lymphocyte blast transformation test on blood samples of 75 individuals, including those with diabetes and sickle cell anemia.

3. A double-blind study involving 20 human subjects showed DMG to be effective in stimulating both a humoral (antibody) as well as a cellular-mediated immune response when a pneumovax vaccine was administered as an immune challenge. A 4.5-fold increase in antibody titer was seen in the DMG test group as compared to the control group. The Leukocyte Inhibition Factor (LIF) also increased significantly in those individuals given DMG.

Depending on the situation, DMG can produce multiple effects upon the immune system. The research showed that DMG significantly stimulates B-cells to produce higher antibody responses (humoral branch)[18,19] and to potentiate a greater activity of T-cells and macrophages (cellular immunity branch).[19] In a clinical evaluation, DMG was shown to return below par lymphocyte activity to near normal in patients with diabetes or sickle cell anemia in an *in-vitro* blast transformation assay. Patients with sickle cell anemia and diabetes tend to suffer more infections than do healthy people.

Research completed in the latter part of 1986 in our laboratory at Clemson University, confirmed that DMG does indeed potentiate both arms of the immune response system.[20] It was concluded that rabbits given DMG and either a typhoid or influenza antigen produced greater than a 4-fold increase in antibody production as compared to control rabbits. The DMG fed animals also showed from a 3 to 9 times greater increase in T-cell count as compared to controls, depending on the vaccine challenge that was used.

DMG was also found to cause a two-fold increase in interferon (IFN) production in rabbits. Interferon is a cytokine, a product of T-lymphocytes, which shows antiviral and anti-tumor activity. Later work using hybridoma cells and other cell culture models revealed that DMG could affect the production of a number of cytokines that can affect both inflammatory processes and tumor growth.[6-9] Cytokines are proteins produced by immune cells that regulate inflammation and modulate the activity of both B and T cells in the immune response. They function as the immune system's communication molecules that act to keep the immune response in balance and functioning properly.

DMG was investigated in a number of bioassays in our lab to evaluate the modulation of cytokine expression in various cell lines. Supernatants of DMG, treated and untreated cytokine producing cell lines (producers), were added to indicator cell lines specific for the production of a respective cytokine. Proliferation or inhibition of these indicator cell lines signals the extent of cytokine secretion by the producer cell lines. THP-1 cells, which secrete TNF-α (Tumor Necrosis Factor-Alpha), were treated with varying concentrations of DMG. Supernatants from these cells were then added to the indicator cell line (L929), which are sensitive to TNF-α. As the concentration of TNF-α increased, there was a concomitant decrease in viability of the indicator (L929) cell line. The viability of L929 cells was measured and the data are presented in Figure 2. As the concentration of DMG increased from 1mM to 100mM, TNF-α secretion levels increased proportionally as detected by the decrease in viability of the L929 indicator cell line.

Similarly, U-937 cells, which secrete Interleukin-1 (IL-1), were treated with DMG and the supernatants added to the sensitive cell line A375.S2 (see Figure 3). Again, as the concentration of DMG increased from 1mM to 100mM, the level of IL-1 secretion increased proportionally as detected by the decrease in viability of A375.S2. Comparable results were obtained on treatment of Jurkat E6-1 cells, which secrete interleukin-2 (IL-2), with DMG. Supernatants were added to the CTLL-2 cell line, which responds to IL-2, by enhanced proliferation.[7] (See Figure 4) In summary, as DMG concentrations increased, levels of TNF-α, IL-1 and IL-2 also increased for these cell lines. The DMG induced increase in cytokines, such as TNFα, greatly enhances immunity to infections as well as increased killing of cancer cells. Dosages of DMG at even 10 times the recommended levels do not trigger induction of abnormally high cytokine levels which may lead to fever and produce an increase in inflammation.[8-9]

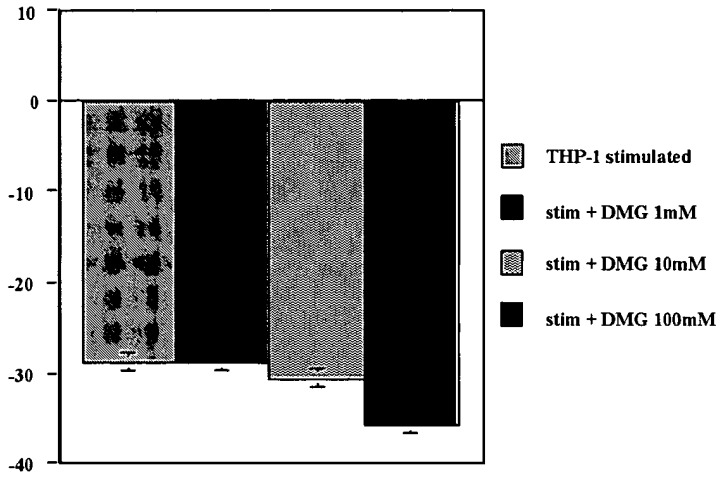


Figure 2. Increase in TNF-α secretion of THP-1 cells upon stimulation with DMG as observed in the L-929 bioassay. Dose response effects of Dimethylglycine (DMG) on TNF-alpha production of LPS Stimulated THP-1 cells. Each data point represents a mean of three independent experiments with triplicate wells.

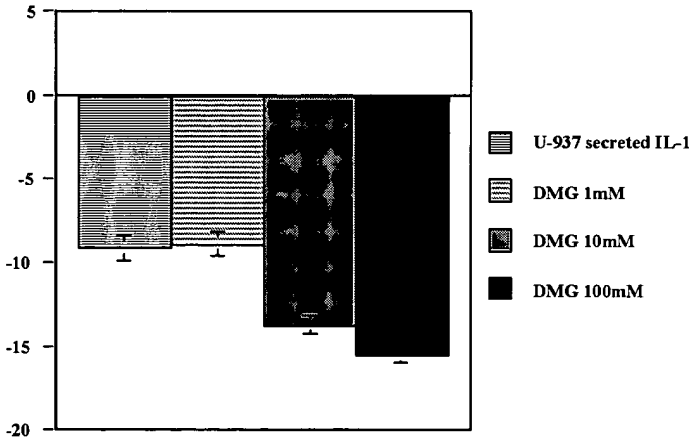


Figure 3. Increase in IL-1 secretion of U-937 cells upon treatment with DMG as observed in the A375.S2 bioassay. Dose response effects of Dimethylglycine (DMG) on IL-1 production of U-937 cells. Each data point represents a mean of three independent experiments with triplicate wells.

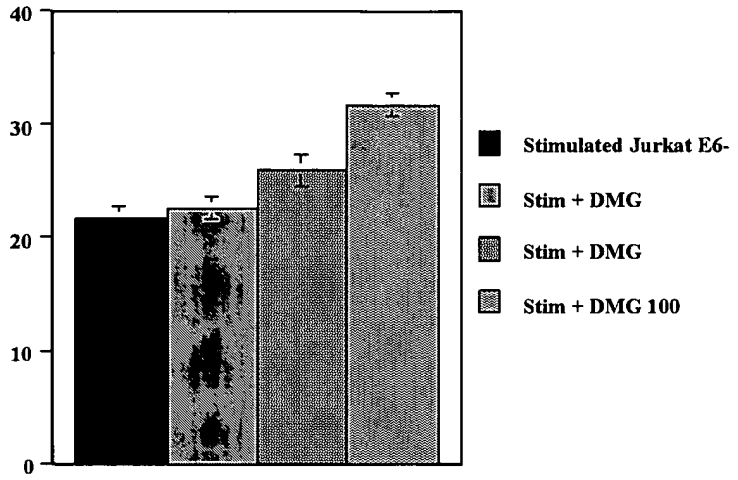


Figure 4. Increase in IL-2 secretion of Jurkat E6-1 cells upon treatment with DMG as observed in the CTLL-2 bioassay. Dose response effect of Dimethylglycine (DMG) on IL-2 production of PMA and Ionomycin stimulated Jurkat E6-1 cells. Each data point represents a mean of three independent experiments with triplicate wells.

An immune study done by the U.S. Army by Bruce Ivins, Ph.D. at the Medical Institute of Infectious Disease at Ft. Detrick, Maryland, evaluated the effects of DMG on guinea pigs given an anthrax MDPH-PA human vaccine.[21] No significant increase in antibody titers were seen in the DMG fed animals as compared to the vaccinated controls. However, when the animals were subsequently challenged with a potentially lethal dose of virulent anthrax bacilli, 4 out of 9 (44%) of the vaccinated control animals died. Among the 11 vaccinated animals given DMG, none succumbed to the lethal dose of anthrax. These remarkable findings seem to indicate that although no increase in antibody titers were seen in the DMG fed animals, as compared to the controls, the DMG fed animals demonstrated an enhanced immunity that must have been due to an enhanced cellular response. This response would correspond to that induced by a sub-population of T cells called Th1 cells, which govern the cellular (T cell) response preferentially over the humoral response (antibody production) governed by Th2 cells.

The use of DMG in humans and animals offers a safe oral immune enhancing nutrient, which can offer increased resistance to and recovery from infectious diseases. Upper respiratory problems, in animals, respond well to DMG supplementation; according to veterinarians who have used the product in their practices. These results indicate that people who use DMG may have greater protection from bacterial and viral infections (flu, colds). This is very important for the geriatric population who may not even respond favorably to vaccinations, because of a weakened immune system. Individuals with cancer, heart disease, allergies, chronic fatigue syndrome or diabetes generally have a compromised immune system and could benefit from supplemental DMG to enhance their immune response to various immunological challenges.

DMG has been shown to impact the Immune System in the following areas:

1. Enhances cellular-mediated response of:
   - B-cells
   - T-cells
   - Macrophages
2. Enhances antibody production
3. Modulates Cytokine production
   - Interferon (IFN)
   - Tumor Necrosis Factor-alpha (TNF-α)
   - Leukocyte Inhibitory Factor (LIF)
   - Interleukin-1 (IL-1)
   - Interleukin-2 (IL-2)
   - Interleukin-6 (IL-6)
   - Interleukin-10 (IL-10)

Melanoma Studies

Work done by our group at Clemson University also strongly suggests that DMG may give an anti-tumor response as a result of its ability to modulate the immune response. Melanoma is a highly metastatic form of skin cancer that spreads easily to other organs. In an initial study using a B16 melanoma model, mice receiving DMG had a significantly higher antibody count, against the B-16 melanoma antigen, as compared to controls. The growth of their tumors was also significantly retarded. The DMG mice lived longer than the controls and had fewer palpable tumors. One of the more important findings, however, was that DMG inhibited or prevented the primary tumors from spreading (metastasis) to vital organs. Histological examination of organ tissue from the control and test mice showed that metastasis to vital organs occurred only in the B-16 control group that did not receive DMG. At the end of the study, all of the control mice had died; of the mice who received DMG supplementation, 71% survived.[22,23]

In a subsequent follow-up study, 48 C57BL/6 mice were placed in five groups. Group 1 consisted of 10 control animals which were inoculated at the base of the tail with B16-F10 melanoma cells which are constitutive metastatic to the mouse lung. The second and third groups, of 5 animals each only, received either water or 50mM DMG in water daily; respectively. These animals did not receive melanoma cells. The fourth study group, of 14 mice, was also inoculated at the base of the tail with the melanoma cells 5 days after the initiation of the daily administration of 50mM DMG in water. Finally, a fifth group of 14 animals were similarly inoculated with the B16-F10 cells 5 days prior to receiving DMG. Again, DMG treatment was continued till the termination of the study. After 28 days all animals were euthanized and scored for primary tumor development at the inoculation site and the extent of metastasis to lung, liver, kidney and heart.[6,7]

Table 3 shows the effect of DMG on primary tumor and monastic foci development. Twenty eight percent of animals receiving daily dosages of 50mM DMG in their water prior to melanoma inoculation, and 50% of mice similarly dosed with DMG beginning five days after inoculation, developed primary tumors as compared to 70% in the controls. More importantly, only 14% of mice pre-treated and 21% of mice post-treated with DMG developed metastatic foci, as compared to 50% in non-DMG controls. The control animals given only water and DMG were normal. These results confirm that DMG significantly reduced the formation of tumors and prevented their spread to other sites in the body.

Table 3: Effect of DMG on the Induction of Both B16 Mouse Melanoma Primary Tumors and Metastic Foci on C57BL/6 Mice

Treatment	# of Animals	% Primary Tumors	% Metastasis
B16 control	10	70%	50%
DMG control	5	0%	0%
Water control	5	0%	0%
DMG pre B16	14	28%	14%
DMG post B16	14	50%	21%

In this model, DMG was administered 5 days prior to exposure of the immune system to the melanoma cells to allow time for equilibration of the nutrient and "priming" of the immune system. This regimen would then represent a prophylactic use of DMG. On the other hand, the initiation of DMG feeding 5 days after melanoma inoculation would allow time for early development of the tumor and/or its spread prior to DMG modulated immune exposure. Since mice usually die about 30 days after melanoma inoculation with rampant tumor and metastatic growth, the 5-day interval would constitute about 1/6 of the expected survival time of the animals. The administration of DMG to these mice would then represent a therapeutic regimen.

Future studies are planned in order to determine at what stage in the development of the tumor and metastatic foci during the 30-day period that the feeding of DMG will still be efficacious. Furthermore, since these data result from daily administration of only 50mM DMG, new studies will also concentrate on determining the most effective daily dosage of DMG required to better control tumor development and prevent metastasis. Higher doses may prove to be more effective. Based upon safety studies by Meduski, there is little concern that higher dosages of DMG would exert a toxic effect.[11]

Previous *in vitro* studies in our laboratory (Figures 3-4), indicated that increased dosages of DMG enhanced secretion of anti-tumor cytokines such has TNF-α, IL-1 and IL-2. These *in-vitro* results may suggest a possible mechanism of action of DMG against melanoma in the mice.

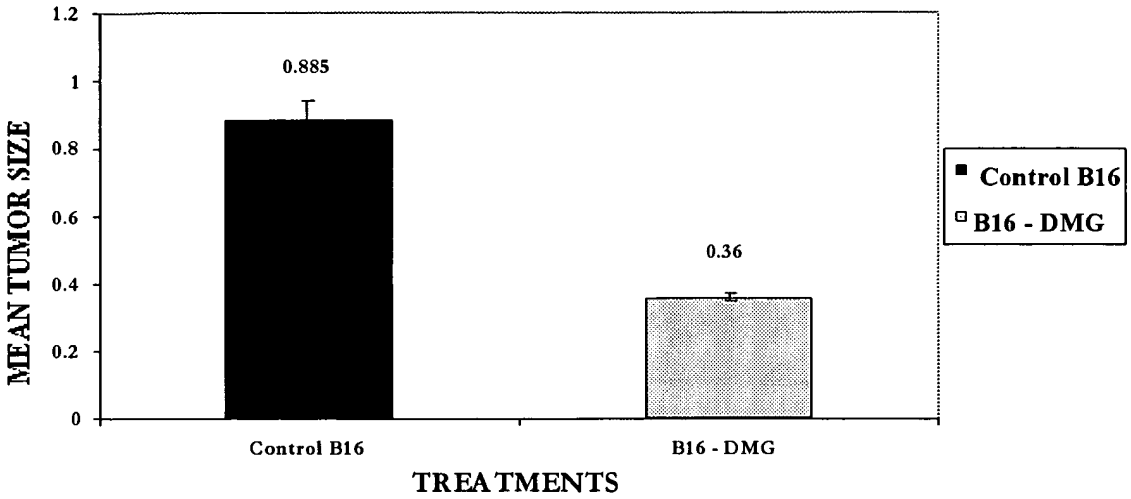


Figure 5. B16 Tumor Formation on the Chlorioallantoic Membrane of Chick Embryo. Tumor formation on the chlorioallantoic membrane of chick embryos by the DMG treated and control mouse B16 melanoma cells. Each data point represents a mean of three independent experiments

DMG also exerted a strong anti-tumor effect against melanoma in a CAM (chlorioallantoic membrane) chick embryo assay. In this assay, DMG reduced tumor growth by 60% as compared to controls. This second test method reinforces DMG's potential clinical use against tumors.[8,9]

Melanomas in general are not easily recognized by the immune system since the level of major histocompatibility complex (MHC) expression on their cell surface is low. MHC expression is central to the function of immune recognition. Figure 6 shows that DMG treatment of peripheral blood leukocytes increased MHC expression. This expression is time and dose dependent. At 72 hrs post-treatment with DMG an increased expression of MHC was measured by an increase in mean channel shifts as determined by flow cytometry. This shift is associated with a 29% increase in the percentage of cells expressing MHC-I, which govern cellular immunity. This increase in MHC expression may indicate enhanced detection of the usually poorly recognizable melanoma cells by the immune system.[8,9]

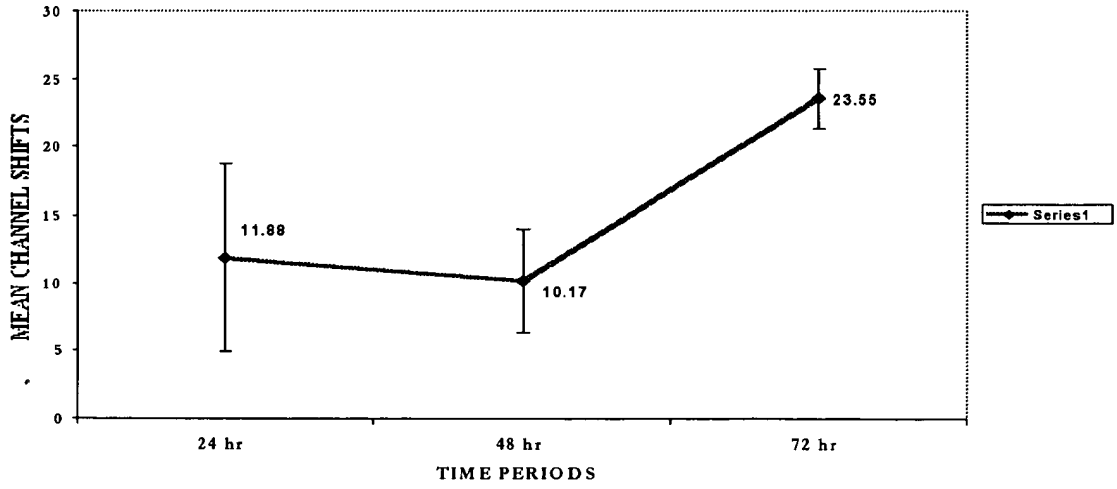


Figure 6. Increase of MHC-I Expression in Peripheral Blood Mononuclear Cells over Time. Mean channel shifts in MHC-I expression of DMG treated peripheral blood mononuclear cells over untreated control at different time periods. Each data point represents a mean of three independent experiments.

Other preliminary studies indicate that DMG may give important nutritional support to the immune system against other forms of cancer, including breast tumors. FoodScience Corporation is continuing to fund studies in this important area.

An Animal Case Study

There have been several reported examples where DMG may have demonstrated activity against tumors in dogs or horses as reported by their owners. One such case was reported on by Hans Kugler, Ph.D., in the *Preventative Medicine Up-Date* (1990) regarding his 11-year old Shepard dog, Foxie.[24] She was diagnosed with a large abdominal tumor, a possible carcinoma, in late 1987. James Jensen, DVM, made the diagnosis and recommended immediate surgery. An ultrasound along with blood tests indicated that the tumor was very large and had probably already spread to the liver and kidneys. Due to these findings, Dr. Kugler decided against surgery and instead put the dog on a natural anti-tumor and immune enhancing program including DMG and an extracted product of spirulina and dunaliella algae called Phycotene. He also included a basic vitamin and mineral supplement along with beef liver concentrate.

Foxie was put on a daily regimen of 250 mg of DMG from FoodScience Corporation and the equivalent of 100,000-150,000 IU of vitamin A from the phycotene. Starting from a very weakened state, Foxie began to improve after only one week on the program. Her energy levels and sleep pattern improved and the tumor mass became noticeably softer upon examination. After six weeks the tumor had shrunk to nearly half its original size and continued to decrease in size until the twelfth week when it was then barely palatable. Later examination by Dr. Jensen revealed that the tumor was completely gone. Foxie had returned to her own usually active state and no longer had any symptoms from the disease. Her remarkable recovery was based on the use of a combined nutritional therapy including DMG. Since other factors had been used, the role that DMG played in reducing the tumor is not exactly known. Based on the most recent findings where DMG showed anti-tumor activity in three specific test protocols, including its ability to increase cellular production of TNF, one can surmise that DMG probably had a significant role in Foxie's recovery. Only after the completion of a well controlled study in animals will a more complete picture of DMG effectiveness against tumors be known.

Lupus Study

Systemic Lupus Erythematosis (SLE) is a complex disorder of the immune system where the body's own immune system begins to attack itself. It usually affects multiple systems of the body and the cause for the disease is not fully understood. It may involve genetic and environmental factors. Treatment focuses primarily on dealing with the many symptoms of lupus, including the use of cortisone drugs (Prednisone). Symptoms include fatigue, fever, arthritic-like joint pain, the typical butterfly rash, inflammation, anemia and photosensitivity to name a few. It is characterized by the presence of autoantibodies to nuclear, cytoplasmic and cell surface proteins.[25]

An accepted and documented model of SLE is the MRL-lpr mouse model. The Clemson group used this model to first obtain a baseline immune cell (CD4+, CD8+, B cell) count and then treat the animals with DMG, Perna canaliculus (green-lipped mussel) and a DMG-Perna combination in order to study a potential role for these in alleviating symptoms of SLE. The baseline study showed that the SLE animals had increased levels of C8+ (cytotoxic cells) and decreased levels of CD4+ (helper cells) in comparison with normal parental Balb/C mice. Results showed that both the DMG and Perna-DMG combination treatment groups restored the CD4+ cell counts to normal and even marginally increased them while decreasing the CD8+ population. These results are indicative of a possible restoration phenomenon. Inflammatory cytokines are again involved in the SLE syndrome and treatment with Perna or the Perna-DMG combination significantly decreased IL-6 levels. There was also a decrease in IL-10 levels in the Perna-DMG group. This is indicative of anti-inflammatory activity by this combination. Preliminary data from the analysis of nuclear (DNA) autoantibodies show that in the DMG-Perna combination group anti single-stranded (ss) DNA antibody levels initially rose along with the controls. However, by the 8th week of treatment, anti-ss DNA antibody titers leveled off and by the 9th week were significantly lower than that observed in the untreated controls (see Figure 7). This decreased level of antibody expression persisted and became even more pronounced, compared to controls, until the study was terminated by the 11th week. Similar results were observed on measurement of antibodies to double-stranded (ds) DNA.[7,8]

In summary, the results of the experiment were totally unexpected; in that DMG and Perna used by themselves made no significant differences compared to the control group. However, when DMG and Perna were used together, the following results were achieved:

- Significant reduction of the Cytotoxic T-cells (CD8+).
- Significant reduction of the regulatory cytokines IL-6 and IL-10. (The role of IL-10 in the induction of autoantibodies in human SLE has been recently suggested.)
- Increased levels of Tumor Nucrosis Factor alpha (TNF-α). Lupus mice have exceptionally low levels of TNF-α and replacement therapy with TNF-α significantly delays the development of kidney damage.
- Significant decreases in nuclear autoantibodies to both single and double stranded DNA. The decrease in serum levels of IL-10 could be associated with this observation.

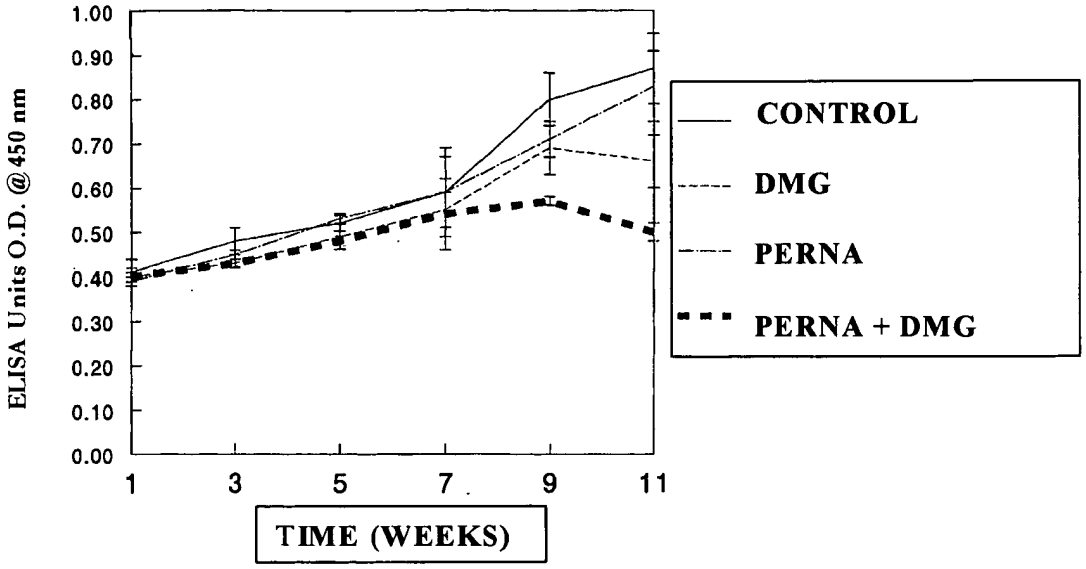


Figure 7. Effect of DMG, Perna and Perna-DMG combinations on serum anti-ss DNA titers in MRL-lpr mice

This experiment provided substantial evidence as to the synergistic combination of Perna and DMG as a possible treatment protocol for SLE. Further studies are now underway to substantiate whether this combination would also be effective in treating human SLE (Lupus).

Case History of a Lupus Patient

Anne MacKenzie of Covington, Louisiana, was diagnosed as having Lupus by her Rheumatologist in early 1999.[26] She suffered from extreme fatigue, a high SED rate, high RNP and antinuclear antibodies, and extreme pain in her tibia and in the joints in her legs. The doctor prescribed Prednisone, 20 mg daily, and rest. Beginning in June, she began to take DMG (750 mg) and Perna mussel (4,000 mg) daily in divided dosage. After several weeks on the new program, she began to experience some relief from the severe pain in her legs and greater exercise tolerance. After three months her doctor reduced Anne's cortisone levels and her lab result improved significantly. The SED rate was improved and the high antibody titers to antinuclear antibodies had returned to normal. After six months, the patient has better energy, her leg and joint pain has all but disappeared and her Prednisone dosage has been reduced further to 5 mg daily. She and her doctor are delighted in her progress and she largely attributes her improved condition to the DMG and Perna program she has been taking faithfully over the past six months.

Cardiovascular System

DMG supplementation has been found beneficial for circulatory problems, high blood pressure and coronary heart disease. DMG enhances oxygen utilization to the heart during hypoxia (low oxygen availability), reduces angina pain, and improves other characteristics of the blood, including reduced cholesterol and triglyceride levels.

According to a symposium conducted in Moscow in 1964 (USSR Academy of Sciences and the Institute of Biological and Medical Chemistry), the principal field of DMG application in trials conducted at over 20 clinics was in the treatment of cardiovascular disorders. The DMG formula was marketed as calcium pangamate, but careful assessment of the patent and scientific literature has demonstrated that Dimethylglycine was the active ingredient in the orally administered product.

The studies reported major improvements in the areas of arteriosclerosis, atherosclerosis, coronary circulation and myocardial function, angina pectoris and high blood pressure upon administration of DMG. Good results were obtained on daily doses of DMG from 50 mg to 100 mg. The principal mode of action to which the improvements were ascribed centered on DMG's ability to decrease hypoxia to the various tissues, to improve various oxidative processes in the body, and to normalize lipid and carbohydrate metabolism.

A four-year clinical evaluation of DMG by Mitchell Pries, M.D. of Palmdale, CA has confirmed the Soviet findings. In trials involving the administration of DMG (125 mg BID) to over 400 cardiovascular patients;[2, 27] Dr. Pries reported major improvements in the following areas:

- Mood
- Circulation to the extremities
- Cholesterol and triglyceride levels
- Angina pain
- High blood pressure
- Arrhythmias
- Stress tests

The patients were on a reduced fat diet and were maintained on their normal medication. Most of the patients in the study had elevated cholesterol levels of greater than 250 mg/dl. After taking 250 mg of DMG for 3 months, most showed a 19 percent drop in total blood cholesterol and a major drop was seen for the triglycerides as well. The patients underwent standard diagnostic evaluations including blood chemistry, electrocardiograms, stress tests and doplar blood vascular readings. Dr. Pries concluded that 125 mg of DMG, taken twice daily, was effective in producing significant improvement in his cardiovascular patients and allowed many to reduce their medication levels. He suggested that higher doses of DMG might yield a faster improvement in his patients.[27]

Sports Practice and Athletic Use

The value of DMG as a nutrient and metabolic enhancer was first observed in the area of athletic performance. DMG has long been used by athletes to improve overall performance and endurance, to enhance oxygen utilization, and to improve recovery of muscles after strenuous exercise. It has been shown in a number of athletic and animal studies over the past 20 years that DMG specifically improves stamina and cardiovascular function in the body.[28-30]

Thomas Pipes has reported in the *Physicians and Sports Medicine Journal* that DMG significantly improved performance in a group of track and field athletes.[2] The DMG group showed an increase of 27.5% in VO_2 Max and of 23.6% in time to exhaustion as compared to the placebo group. DMG's apparent ergogenic (energy enhancing) effects could be due to a number of factors, including better oxygen utilization and cellular respiration, reduced lactic acid build-up, and enhanced carbohydrate and lipid metabolism.

A 1982 study by Levine and Myhre published in *Equine Practice* showed DMG's ability to reduce lactic acid level in exercised Standardbreds as compared to controls. The authors reported over a five-fold decrease in lactate level buildup for the DMG fed animals as compared to the controls.[31]

Robert Cator, DVM, of the Panhandle Regional Veterinary Clinic, confirmed the earlier results of Levine; and Myhre using Thoroughbreds under more controlled conditions. Thoroughbreds were monitored for speed and blood lactate levels with and without DMG at a constant level of 210 heartbeats per minute. The results show greater speed with a lower blood lactate level when DMG was part of the horse's diet.[32]

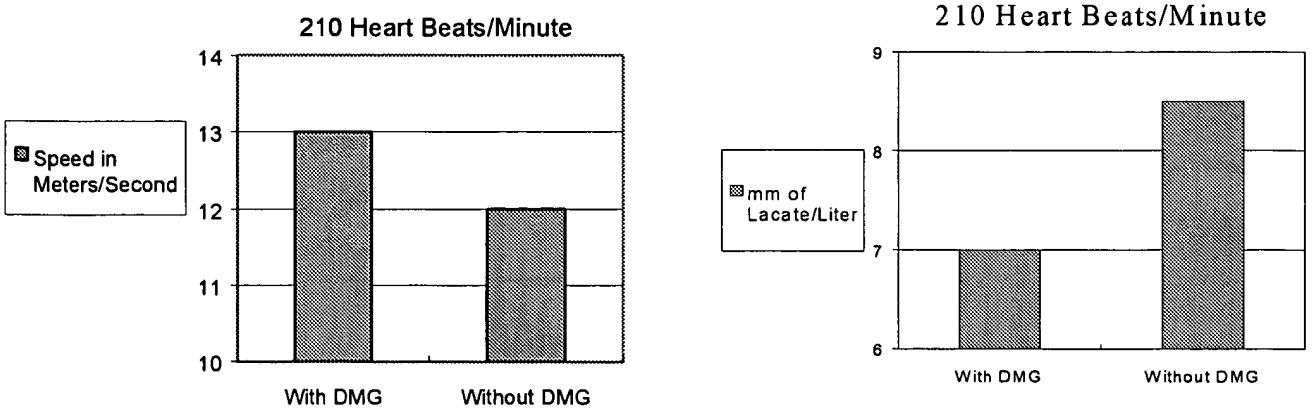


Figure 8. The Effects of N,N-Dimethylglycine on Blood Lactate Levels and Speed Racing Thoroughbreds

--Field work conducted at training track at Panhandle Regional Veterinary Clinic in Spearman, Texas, by R. L. Cator, Jr., DVM

--Laboratory work performed by Texas A&M Medical Veterinary Diagnostic Laboratory, Amarillo, Texas by John Halliburton, Ph.D.

Potter and Moffitt of Texas A&M reported an equine lactic acid lowering effect using a treadmill method. When horses were supplemented with DMG at the rate of 1.6 mg/kg, they had lower blood lactate concentrations during strenuous exercise as compared to when unsupplemented. This dosage equates to approximately one serving of 750 mg of DMG for a 1,200-1,400 lb horse.[33]

The actual mechanisms of how DMG improves oxygen utilization and reduces lactic acid accumulation are not clearly understood, but its effectiveness in improving performance and reducing recovery time after strenuous exercise is well-established. In a paper published in *Canine Practice*, Cannon and Kendall reported that racing greyhounds had improved racing times and better recovery times when supplemented with DMG.[34]

Meduski and co-workers at the University of Southern California conducted experiments with rabbits to monitor the effect of Dimethylglycine on lactic acid production under hypoxic conditions. New Zealand white rabbits were exposed to severe surgical stress; a condition known to increase blood lactate in animals. Those animals that received DMG showed markedly decreased levels of lactic acid in their blood as compared to controls.[11]

Meduski also reported improved oxygen uptake of rats supplemented with DMG and exposed to a low oxygen (8%) environment. The DMG fed rats showed better adaptation to hypoxia than did the controls by living 80% longer under a low oxygen environment as compared to controls.[11]

The New York City marathon club, under the direction of Gary Null, Ph.D., did a six-month study on the use of DMG supplementation in marathon runners. The evaluation involved comparing the running times, recovery period and fatigue levels to a previous race where DMG was not used. In the second race the runners took 500 mg DMG before the start of the race and then followed by taking 250 mg for every four miles covered for a total of 2000 mg of DMG. The improvements were noteworthy. Marathoners reported much less exhaustion as compared to the previous race. Based upon compiled statistics, better race times were obtained along with improved stamina, muscle and body recovery, and especially the reduction of cramping and fatigue from the 18th to the 26th mile mark in the marathon.

The animal studies, along with actual field evaluations, show that DMG may be beneficial to endurance athletes (runners, team sports) as well as short-timed events (weight lifters, sprinters). Not only will DMG help in overall performance and recovery, but it will also boost the immune defense of the individual athlete and make him/her less susceptible to infections or other stress related illnesses.

Neurological and Brain Support

A lack or imbalance of neurotransmitters can cause a whole series of brain and nerve dysfunctions. Because DMG can act as a precursor to a number of amino alcohols and acids which aid brain function, including glycine, its value to the body's neurological system should not be overlooked. There is considerable support that DMG can function as a safe and effective anticonvusant/antiseizure agent.

Dr. William Freed conducted an extensive experiment in Swiss-Webster mice where he compared the prevention of strychnine-induced seizures by glycine and its three N-Methylated derivatives betaine, DMG and sarcosine.[35] All three methylated glycines, betaine, DMG and sarcosine inhibited strychnine-induced seizures and death. Glycine had no effect. Betaine, DMG and sarcosine reduced, respectively, deaths by 76, 65 and 45%; seizures by 61, 55 and 39%, all at a dosage of 5 mM/kg. Freed speculated that DMG's anti-convulsant property may be linked to a yet unrecognized disorder of sulfur-amino acid metabolism.

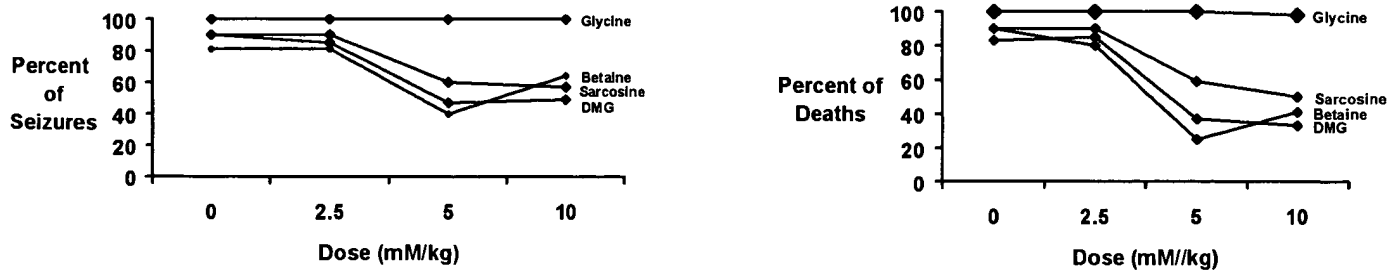


FIGURE 9: Dose Response Effects of Glycine and the N-Methylated Derivatives of Glycine in Reduction of Seizures and Death Induced by Strychnine. Percentages of animals manifesting seizures and death following administration of 2.5 mg/kg of strychnine as a function of betaine, DMG, sarcosine, and glycine dosage. (a) Percentage of animals with seizures. For the 5 mM/kg dosage, the decrease in seizure frequency as compared to saline (0 mM/kg) was statistically significant for betaine ($p=0.011$), DMG ($p=0.001$) and sarcosine ($p=0.009$), but not for glycine (Fisher's exact test). (b) Percentage of deaths. For the 5 mM/Kg dosage, the decrease in percentage of deaths was statistically significant for betaine ($p=1.27\times10^{-4}$), DMG ($p=7.72\times10^{-4}$), and sarcosine ($p=0.016$), but not for glycine. Between 20 and 22 mice were tested at each dosage point. From Freed, W., *Pharm, Biochem and Behavior* (1985).

In another study, Thomas Ward, M.D., found that DMG significantly reduced mortality associated with penicillin-induced seizures in rats. There were 36 rats each in the control group and in the DMG administered group (fed 2.0 mg/kg DMG daily in their drinking water for 2 weeks). There was a substantial difference in survival between the two groups given high levels of penicillin G. Only one rat in the DMG-treated group died, compared with 18 in the control group. Ward linked DMG seizure protective effects to a possible effect on increasing glycine levels in the DMG fed rat; or that DMG may be acting directly on receptor sites in the central nervous system (CNS).[36]

Drs. Roach and Carlin, of the Bowman Gray School of Medicine, reported in the *New England Journal of Medicine* a case history where DMG was effective in controlling epileptic seizures in a 22 year old man who had a long history of mental retardation and mixed complex, partial and gran mal seizures. Before treatment the patient was experiencing 16 to 18 generalized seizures per week, even when on therapeutic levels of phenobarbital and carbamazepine. After taking 90 mg of DMG twice daily for one week, the number of seizures was reduced to three per week. Two attempts to eliminate DMG supplementation resulted in a dramatic increase in seizure frequency for the individual.[4] The monitored improvement on other epileptic patients has not been as dramatic as in the case cited, but several other instances of DMG aiding in seizure patients have been report by Gasion and Patterson.[37] Veterinarians have reported good success in preventing seizures in dogs and cats using DMG.

Autism

There is a growing body of evidence that DMG is beneficial for individuals with autism. Autism is a biological brain disorder of unknown causes that results in a wide range of puzzling and disturbing social and personal behavior patterns. Two studies and many hundreds of clinical case reports have demonstrated that DMG can modify and improve the behavior, social interaction, verbal communication and disturbing activities of autistic children. Significant improvements were seen in many areas.

The first study was conducted in 1990, by Dr. Lee Dae Kun, Director of the Pusan Research Center on Child Problems in Korea. In this study, as reported in the *Autism Research Review*, 39 autistic children ages three to seven, were given 125 mg to 375 mg of DMG per day, depending on weight, for a three-month open trial period.[5] Based on the evaluation of both parents and teachers in a number of key areas, 80% of the children improved significantly.

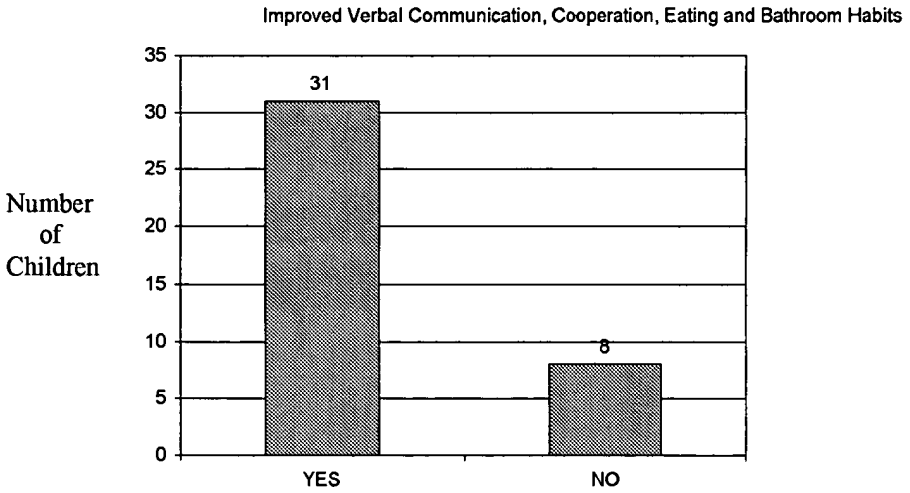


Figure 10. Resulting Benefits of Dr. Kun's Study

Several children had difficulty sleeping and were more active during the first few weeks, but this situation improved as the study progressed. The improvement in the children was especially noted by the parents.

A second study was completed in 1997 in Taiwan by Dr. Shin-siong Jung of the Taipei Springtide Foundation. Unlike the Korean study, this was a double-blind placebo controlled study involving 84 autistic children who were divided into two groups. Forty-six children were placed in a DMG test group, while 38 children were placed in a placebo group. The DMG test group showed statistically significant improvement on all five Aberrant Behavior Checklist scales used to evaluate effectiveness: irritability, lethargy, stereotypy, hyperactivity and inappropriate speech. The placebo group showed improvement only on the lethargy scale.[38] This study will soon be published.

The results from Taiwan confirm the hundreds of communications that the Autism Research Institute, in San Diego, CA under the direction of Dr. Bernard Rimland, has received from delighted parents who have seen remarkable changes in their autistic children after using DMG. Dr. Rimland has recommended the use of DMG for autism since 1980. Quoting from his *Autism Research* Review he writes, "A number of parents have reported that within a few days of giving their children one 125 mg tablet of DMG per day, the child's behavior improved noticeably, better eye contact was seen, the child's speech improved, or more interest and ability in speaking was observed." He now recommends from 1 to 4 125 mg tablets per day depending on the child's age but some parents have used much higher levels with good results. For adults, the usual dose is eight tablets per day. A California psychiatrist and mother wrote Dr. Rimland the following letter:

> ***"I am extremely excited by the news about my 23-year old autistic son, Bruce, since he has been on DMG. Today is his ninth day on DMG, and his third day on 375 mg/day. As you can imagine, we have tried every conceivable remedy and method known to man for the last 23 years. Bruce has stopped yelling and screaming and biting his arm. He is quiet (not listless) and seems happy and contented. He no longer performs his maddening acts of perserveration-the ritualistic and compulsive rituals that consumed hours every day and drove us crazy. On Sunday, he sat and watched the Super Bowl on TV. He has never watched TV before in his life. Because of his many compulsive rituals, it used to take him almost two hours to get dressed in the morning. Now he gets dressed in five minutes. His eye contact has improved and he walks around with a happy smile on his face."***
>
> ***A.K., MD***
> ***Thousand Oak, CA***

In another letter to the Autism Research Institute, a woman reported that DMG had a profound effect on reducing her brother's seizures and improving his verbal communication.

> ***My brother has fragile X syndrome, mental retardation, and autism. He has been plagued with seizures for many years, often having several minor motor seizures a day. After leaving the hospital recently, he continued to have seizures. He was still getting phenobarbital, mysoline, and dilantin. I called the Autism Research Institute (ARI) and found out about DMG. I immediately bought some and sent it to his facility. They gave it to him. He has not had a single seizure since. It has been over three weeks. Ever since my brother started taking the DMG (125 mg two times a day), he has been talking a blue streak. He is talking about a variety of subjects and is using more phrases and sentences. He even told a nurse, "I told you no. N-O!" He has never spelled anything before. He is also counting. He is happy as a lark. He is better able to control himself if something upsets him.***
>
> ***K.Z.***
> ***Menlo Park, CA***

Areas where parents and teachers have noted improvement with DMG with autism include:

- Better Verbal Communication
- Better Eye Contact
- Improved Affection
- Better Social Interaction
- Reduction in Seizures

There are a number of possible explanations as to why the use of DMG has resulted in such remarkable improvement. These include better oxygen utilization, reduction of lactic acid formation and a possible decrease in potential seizure activity. Perhaps the most relevant research on DMG relative to autism may be in the immunological area. Recent publications have shown that certain components of the immune system may be abnormal in people with autism. These include a decreased number of helper T-cells and B-cells, reduced natural killer cell activity, inhibition of macrophage activity and increased interferon levels. DMG's influence on the immune system may correct the immune system defect of autistic children responsible for the prevalent symptoms. Further studies in this area are needed to further elucidate the specific role DMG may play in reversing autism.

Safety

DMG has been found to be an extremely safe food substance as demonstrated by a series of animal studies conducted at the Medical University of Southern California. Meduski has reported that Dimethylglycine Hydrochloride has an LD_{50} (lethal dose to 50% of the animal population) of 7,400 mg per kg of animal body weight in the rat. This amount is generally regarded as nontoxic.

DMG is a water-soluble nutrient and the enzyme system in the body effectively converts the substance into metabolites that are either used by the body or are safely excreted from the body. Feeding studies at reasonably high levels have demonstrated DMG's long-term safety. A two-year feeding study in rats produced no health problems, even when fed at a level 1/10 the LD_{50} on a daily basis (740 mg/kg of body weight per day). DMG is a safe food substance that has been used by thousands of clinicians and doctors for almost three decades without adverse or negative side effects.

Absorption and Dosage

FoodScience Corporation provides DMG in a pure 125 mg sublingual tablet which dissolves rapidly under the tongue. DMG is very effectively absorbed from the digestive tract, including the oral cavity. Sublingual ingestion of DMG provides effective and rapid absorption, the effects of which are frequently evident within 20 minutes after taking the product. All indications are that DMG is not stored in appreciable amounts in the body but is metabolized by enzymes in the liver into one and two carbon units as mentioned earlier.

The recommended dosage of DMG can range from 125 mg to over 1000 mg per day, depending on the area of use. In most cases it is best to take DMG several times throughout the day in order to maintain a more consistent availability to the body. It is best taken between meals to avoid competitive uptake from other amino acids. Individuals with heavy work schedules (stress), athletes or people dealing with a major health problem including active infections or compromised immune systems can benefit from higher intakes of DMG.

DMG Works Synergistically With Other Nutrients

Dimethylglycine is a versatile healing nutrient that can be used to maintain good health, enhance performance and productivity as well as aid the body in the healing and restoration process. This article has focused on the many areas where DMG has been found to be beneficial to the immune response, cardiovascular system, mental and physical performance, enhancement to cellular metabolism and to many other specific areas. When DMG is combined with other nutritional and therapeutic food factors, such as Perna canaliculus (Green-Lipped Mussel) additional synergistic effects may result. DMG can be safely combined with any nutritional or therapeutic product without negative side effects. In their book, *Prescription for Nutritional Healing*, James Balch, M.D. and Phyllis Balch, C.N.C., recommend DMG for over 35 different health problems.[39]

Dimethylglycine is a time-tested, scientifically proven metabolic enhancer that can bring about greater immune protection, vitality and cellular regeneration. Continued research with this nutrient will reveal even more about how DMG can be used effectively to combat the stresses and health problems that affect so many as we advance into the new millennium.

For More Information and Availability of DMG, Contact:

FoodScience Corporation
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Essex Junction, Vermont 05452
Email: rkendall@foodsciencecorp.com
Telephone: 1-800-451-5190
FAX: (802) 878-0549

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Vetri-Science Laboratories of Vermont:	www.vetriscience.com
U.S. Animal Nutritionals of Vermont	www.usanimalnutritionals.com
Pet Naturals of Vermont	www.petnaturals.com

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